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(54) PREPARATION OF POLYMERIC SURFACES

HERSTELLUNG VON POLYMEREN OBERFLÄCHEN

PREPARATION DE SURFACES POLYMERES

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Description

BACKGROUND OF THE INVENTION

5 [0001] It is often desirable to provide the surface of an object with a polymeric coating to protect the surface or to provide the surface with properties of the polymer coating. For example, various paint-like coating compositions are employed to provide the surfaces of metals, wood and the like with thin, protective polymeric films.

[0002] The adhesion of polymeric films such as those described above to the surfaces to which they are applied are commonly largely mechanical. The surfaces often are roughened or otherwise prepared before a polymeric coating is applied so as to increase the degree of mechanical adhesion. Polymers are generally not chemically bonded to the surfaces upon which they are applied, and polymer coatings generally have not been used as coatings for devices which may be implanted in the human body or to devices which come into contact with body fluids during use, such as contact lenses. Coatings for objects such as these should adhere tenaciously to their surfaces even in the presence of body fluids and other liquids.

15 [0003] To improve the adhesion of certain polymer species to supporting surfaces, U.S. patents 4,663,232, 4,311,573, 4,595,632 and 4,589,964 teach that surfaces to be coated must be carefully prepared, as by precoating, using careful, often time-consuming procedures to receive polymer species.

[0004] International Patent Application WO88/02623 describes a method for improving the biocompatibility of a solid surface comprising use of a biocompatible agent and a chemical linking moiety possessing a photochemically reactive group. U.S. Patent 3,826,678 describes grafting polymers or co-polymers to an inert polymeric substrate using high energy electrons, x-rays or gamma radiation.

SUMMARY OF THE INVENTION

25 [0005] We have discovered that polymer molecules and reactive chemical molecules such as monomers and oligomers may be provided with latent reactive groups covalently bonded to them such that when the molecules are brought into bonding proximity with a substrate such as a surface, the latent reactive groups can be energized to form, via free active specie generation, covalent bonds between these molecules and the substrate. The substrate to which the polymer molecules are to be so attached need not be specifically pretreated so as to add to it functional groups to which bonding occurs, and the invention provides a method by which such molecules may be readily attached to untreated substrates of various types.

[0006] The invention relates to a method of providing a substrate having a solid surface with a polymeric coating, the method comprising the steps of (a) providing a monomer, oligomer or other reactive chemical unit, each having covalently bonded to it at least one latent reactive group capable of active specie generation in response to ultraviolet, visible or infrared electromagnetic radiation; (b) contacting the substrate surface with a composition comprising a plurality of molecules of the monomer, oligomer or other reactive chemical unit under conditions which enable the monomer, oligomer or other reactive chemical unit molecules to orient spatially so as to enable the latent reactive groups to come into covalent bonding proximity with the substrate surface; (c) thereafter activating the latent reactive groups by application of ultraviolet, visible or infrared electromagnetic radiation to covalently bond the monomer, oligomer or other reactive chemical unit molecules to the substrate surface; and (d) covalently bonding by polymerization further monomers or oligomers to the monomer, oligomer or other reactive chemical unit molecules covalently bound to the substrate surface.

[0007] A latent reactive polymeric composition can be applied to a substrate such as a surface and covalently bonded to it by application of an external stimulus. The polymeric composition comprises a plurality of polymer molecules each having covalently bonded thereto at least one latent reactive group capable of active specie generation in response to applied external stimulation to covalently bond to the substrate, the polymer molecules being so spatially oriented as to enable their latent reactive groups to come into covalent bonding proximity with a surface or other substrate to which the coating composition is applied. Desirably, the coating composition includes a vehicle carrying the polymer molecules and within which the polymer molecules are permitted sufficient freedom of movement as to enable latent reactive groups of the polymer molecules to be positioned in bonding proximity with the substrate with which the coating composition is applied.

[0008] The invention provides a surface or other substrate bearing a plurality of polymer chains each covalently bonded to it through a residue of a latent reactive group, which latent group was initially capable of active specie generation in response to application of an external stimulus to covalently bond to the substrate; the polymer chains being present in sufficient quantity as to provide the surface or other substrate to which they are attached with one or more characteristics of the polymer.

[0009] The invention relates to a method of providing a surface of a substrate with a plurality of polymer chains covalently bonded to it, the method comprising contacting the substrate with polymerizable reactive units such as mono-

mers or oligomers each having covalently bonded to it a latent reactive group, and externally stimulating the latent reactive group to cause the same to covalently bond to the substrate via active specie generation. To the thus-bonded reactive units are covalently bonded one or more monomers, oligomers via polymerization of monomers or oligomers to provide polymer chains, the resulting chains thus being covalently bonded to the substrate.

DESCRIPTION OF THE PREFERRED EMBODIMENT

[0010] The polymers may be natural or synthetic in origin. Such polymers include oligomers, homopolymers and copolymers resulting from addition or condensation polymerization, and natural polymers including oligosaccharides, polysaccharides, peptides, and proteins. The polymers may include several distinct polymer types, as prepared by terminal or side chain grafting. The polymers of the invention may include cellulose-based products such as hydroxyethyl cellulose, hydroxypropyl cellulose, carboxymethyl cellulose, cellulose acetate and cellulose butyrate, acrylics such as those polymerized from hydroxyethyl acrylate, hydroxyethyl methacrylate, glyceryl acrylate, glyceryl methacrylate, acrylic acid, methacrylic acid, acrylamide and methacrylamide, vinyls such as polyvinyl pyrrolidone and polyvinyl alcohol, nylons such as polycaprolactam, polylauryl lactam, polyhexamethylene adipamide and polyhexamethylene dodecanediamide, polyurethanes, polylactic acids, linear polysaccharides such as amylose, dextran, chitosan, and hyaluronic acid, and branched polysaccharides such as amylopectin, hyaluronic acid and hemi-celluloses. The polymeric species are chosen so as to exhibit one or more properties desired for the surface or other substrate to which the polymer molecules are bonded. For example, it may be desired in some instances to provide surfaces with very hydrophilic properties, in which case polymer species such as hyaluronic acid may be employed. The polymer polyethylene glycol may be employed to repel proteins as from a contact lens surface. Heparin, a polysaccharide, may be used to impart antithrombogenic characteristics, and chitosan may be employed to provide hemostatic properties.

[0011] The physical characteristics of the polymer molecules employed in the present invention are generally derived from the nature of the molecular chains themselves. Thus, polyvinyl alcohol, for example, which bears a plurality of hydroxyl groups and which is generally water soluble, provides hydrophilic characteristics to a surface or other substrate to which it is covalently bonded through the method of the invention. The polymer molecules of the invention desirably are substantially free of biologically active groups that are either added to the polymer molecules after polymerization or that are not normally contained in the precursor monomers or in identical, repeating units of the polymer. The polymer molecules that are employed in the invention desirably have extended chain lengths of at least about 10 Angstroms, preferably at least 25 Angstroms, and most preferably at least about 50 Angstroms.

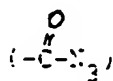
[0012] Most preferably, the polymer molecules include end portions which are free from latent reactive groups and which end portions themselves have extended lengths (measured from the nearest latent reactive group) of at least 10 Angstroms and preferably at least 25 Angstroms, and most preferably at least 50 Angstroms. In this manner, the free polymer molecule end portions may extend as desired away from the surface or other substrate to which the molecule is attached to provide appropriate physical or other characteristics. The polymer molecules themselves preferably have molecular weights of at least about 400, and desirably are generally hydrophilic in nature, the polymers preferably being soluble in water to the extent of at least approximately 0.5% by weight at 25° C. "Extended length", as used herein, refers to the length that a polymer chain would have if it were stretched out to its maximum length, observing proper bond angles between adjacent atoms. Polyethylene glycol, hyaluronic acid, collagen, chitosan, heparin, and polyvinyl alcohol are particularly preferred polymers.

[0013] The polymer molecules employed in the invention carry one or more latent reactive groups covalently bonded to them. The latent reactive groups, broadly defined, are groups which respond to specific applied external stimuli to undergo active specie generation with resultant covalent bonding to an adjacent support surface. Latent reactive groups are those groups of atoms in a molecule which retain their covalent bonds unchanged under conditions of storage but which, upon activation, form covalent bonds with other molecules. The latent reactive groups generate active species such as free radicals, nitrenes, carbenes, and excited states of ketones upon absorption of external electromagnetic energy. Latent reactive groups may be chosen to be responsive to the ultraviolet, visible or infrared portions of the electromagnetic spectrum. Latent reactive groups as described are generally well known.

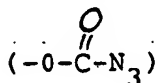
[0014] The azides constitute a preferred class of latent reactive groups and include arylazides



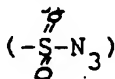
such as phenyl azide and particularly 4-fluoro-3-nitrophenyl azide, acyl azides



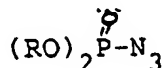
such as benzoyl azide and p-methylbenzoyl azide, azide formates.



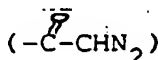
such as ethyl azidoformate, phenyl azidoformate, sulfonyl azides



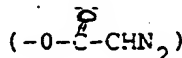
such as benzenesulfonyl azide, and phosphoryl azides



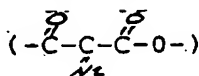
such as diphenyl phosphoryl azide and diethyl phosphoryl azide. Diazo compounds constitute another class of latent reactive groups and include diazoalkanes ($-\text{CHN}_2$) such as diazomethane and diphenyldiazomethane, diazoketones



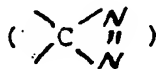
such as diazoacetophenone and 1-trifluoromethyl-1-diazo-2-pentanone, diazoacetates



such as t-butyl diazoacetate and phenyl diazoacetate, and beta-keto-alpha-diazoacetates



such as t-butyl alpha diazoacetoacetate. Other latent reactive groups include the aliphatic azo compounds such as azobisisobutyronitrile, the diazirines



such as 3-trifluoromethyl-3-phenyldiazirine, the ketenes ($-\text{CH}=\text{C}=\text{O}$) such as ketene and diphenylketene and photoactivatable ketones such as benzophenone and acetophenone. Peroxy compounds are contemplated as another class of latent reactive groups and include dialkyl peroxides such as di-t-butyl peroxide and dicyclohexyl peroxide and diacyl peroxides such as dibenzoyl peroxide and diacetyl peroxide and peroxyesters such as ethyl peroxybenzoate.

[0015] Upon activation of the latent reactive groups to cause covalent bond formation to the surfaces to which polymer molecules are to be attached, the polymer molecules are covalently attached to the surfaces by means of residues of the latent reactive groups. Exemplary latent reactive groups, and their residues upon activation, are as follows

	<u>Latent Reactive Group</u>	<u>Residue Functionality</u>
5	aryl azides	amine R-NH-R
	acyl azides	amide $\text{R}-\overset{\text{O}}{\parallel}\text{C}-\text{NH-R}$
	azidoformates	carbamate $\text{R-O}-\overset{\text{O}}{\parallel}\text{C}-\text{NH-R}$
10	sulfonyl azides	sulfonamide $\text{R}-\overset{\text{O}}{\parallel}\text{S}-\text{NH-R}$
	phosphoryl azides	phosphoramidate $(\text{RO})_2\overset{\text{O}}{\parallel}\text{P}-\text{NH-R}$
15	diazoalkanes	new C-C bond
	diazoketones	new C-C bond & ketone
	diazoacetates	new C-C bond & ester
	beta-keto-alpha-diazoacetates	new C-C bond & B-ketocester
20	aliphatic azo	new C-C bond
	diazirines	new C-C bond
	ketenes	new C-C bond
25	photoactivated ketones	new C-C bond & alcohol
	dialkyl peroxides	ethers
	diacyl peroxides	esters & new C-C bonds
30	peroxyesters	ethers, esters, and new C-C bonds

35 [0016] The polymers and oligomers used in the invention may have one or more latent reactive groups. Desirably, the polymers have at least one latent reactive group per molecule with the ratio of reactive groups extended polymer length, in Angstroms, ranging from about 1/10 to about 1/700 and preferably from about 1/50 to 1/400.

[0017] As will be noted from the foregoing description, photoreactive latent reactive groups are for the most part aromatic and hence generally are hydrophobic rather than hydrophilic in nature.

40 [0018] The latent reactive groups and the polymer molecules to which they are bonded may have substantially different solvophilic properties. For example, the latent reactive groups may be relatively hydrophobic, whereas the polymer molecules may be relatively hydrophilic; when solution of the molecules is contacted with a relatively hydrophobic surface, it is believed that the latent reactive groups, being hydrophobic, tend to orient nearer the surface so as to improve bonding efficiency when the latent reactive groups are activated. The preferred latent reactive groups are benzophenones, acetophenones, and aryl azides.

45 [0019] The loading density of polymers upon a surface may be improved by a process in which a latent reactive molecule (a molecule having a latent reactive group) is first brought into close association (as by means of a solvent solution) to a surface, and thereafter the polymer to be bonded to the surface is brought into contact with and is covalently bonded to the latent reactive molecule, as to a reactive group different from the latent reactive group. Thereafter, the latent reactive groups may be activated to cause them to covalently bond to the surface to thereby link the polymers to the surface. This procedure appears to work particularly well when the latent reactive group is solvophilically compatible with (e.g., similar to) the surface and wherein the polymer is relatively solvophilically incompatible with the surface but is more compatible with a portion of the latent reactive group. Reference is made to Example IX below as being illustrative of this procedure.

50 [0020] Polymer chains may be provided upon a surface or other substrate by first covalently bonding to the substrate through a latent reactive group a monomer, oligomer or other reactive chemical unit. To the thus-bonded reactive units are covalently bonded monomers or oligomers in a polymerization reaction.

[0021] The reactive chemical units of the invention carry covalently bonded thereto latent reactive groups as described herein for covalent attachment to a non-pretreated surface or other substrate. These molecules are charac-

terized as having reactive groups capable of covalent bonding to polymer molecules of a polymer having the desired characteristics, or of entering into a polymerization reaction with added monomers or oligomers to produce polymer chains having the desired characteristics. Reactive chemical molecules capable of covalently bonding to polymer molecules include not only monomers and oligomers of various types but also molecules having such functional groups as carboxyl, hydroxyl, amino, and N-oxysuccinimide, such groups being reactive with reactive groups carried by the polymer chain to bond to the chain. The reactive chemical molecules are preferably monomers or oligomers and most preferably are ethylenically unsaturated monomers capable of entering into an addition polymerization reaction with other ethylenically unsaturated monomers. Particularly preferred are the acrylate and methacrylate monomers which are the esterification products of acrylic or methacrylic acid and hydroxy-functional latent reactive groups. Examples of such molecules include 4-benzoylbenzoyllysyl-acrylate.

[0022] Utilizing reactive chemical units bearing latent reactive groups, one will desirably first coat a surface or other substrate with a solvent solution of such molecules. Upon removal of solvent, the application of an appropriate external stimulus such as U.V. light will cause the molecules to covalently bond, through the latent reactive groups, to the substrate. The substrate may then be appropriately contacted with a solution containing the desired monomer or oligomer molecules to cause bonding to these molecules. For example, if the reactive chemical molecule is a monomer or oligomer, e.g., a methacrylate monomer, the substrate to which the molecule is covalently bonded may be contacted with a solution of addition-polymerizable monomers such as hydroxyethyl methacrylate and a free-radical addition polymerization initiator such as dibenzoyl peroxide under addition polymerization conditions to result in the growth of polymer chains from the monomer molecules bound covalently to the substrate. Once the desired polymerization has occurred, the substrate may be washed to remove residual monomer, solvent and non-bound polymer that was formed.

[0023] The term "substrate" refers to any chemical moiety to which polymer molecules are to be attached through activation of latent reactive groups. The substrate may take the form of molecules in a solution, but more desirably, the substrate comprises a definable surface such as the tangible surface of a contact lens or surgical implant, or the surface provided by small particles in an emulsion or other suspension or as a powder, or the surface defined as the interface between two substantially distinct phases, such as two immiscible liquid phases or the surface of a soft gel. Although the polymer molecules may be attached to the same or different polymer molecules in a solution, as described more fully below, the invention provides the particular advantage of providing means by which non-pretreated definable (e.g., solid) surfaces may simply and rapidly be provided with covalently bonded-on polymer coatings in a simple, rapid and hence economical manner.

[0024] "Hydrophilic" and "hydrophobic" are used herein to describe compositions broadly as water-loving and water-hating, respectively, in line with the following observations: Hydrophilic compounds are usually relatively polar and often are ionizable. Such compounds usually bind water molecules strongly. Hydrophobic compounds are usually relatively non-polar and non-ionizing. Hydrophobic surfaces will generally cause water molecules to structure in an ice-like conformation at or near the surface. "Hydrophobic" and "hydrophilic" are relative terms, of course, and are used herein in the sense that various compositions, liquids and surfaces may be hydrophobic or hydrophilic relative to one another. A discourse on the subject is found in Hoffman, Letter to the Editor: A General Classification Scheme for "Hydrophilic" and "Hydrophobic" Biomaterial Surfaces, J.Biol. Mat. Res. 20, pp ix-xi (1986).

[0025] The loading density resulting from attachment of polymer molecules to a surface or other substrate in accordance with the invention may be regulated in several ways. First, the degree of activation of latent reactive groups is generally a function of the quantity of the external stimulus that is applied, and thus the extent of covalent bonding through the latent reactive groups may be regulated by regulating the intensity and time of application of the applied stimulus. Regulation of the applied stimulus is particularly easy when the stimulus is actinic radiation; one can readily regulate the amount of radiation to which the latent reactive groups are exposed. Loading density may also be regulated by adjusting the capacity of polymer molecules of the invention to bring their latent reactive groups into bonding proximity with a surface. Thus, one may regulate the viscosity of a solution of polymer molecules in an appropriate solvent as well as the solubility of polymer in the solvent. Yet another factor is the concentration of polymer molecules in a coating composition.

[0026] As will be understood from the above discussion and from the examples which follow, the invention permits a substrate, particularly a solid surface, to be provided with covalently attached polymer molecules in sufficient loading density or quantity as to provide an "effective" surface having the physical properties of the added polymer rather than those differing physical properties of the uncoated solid surface. In this manner, for example, the hydrophobic surface of polystyrene may be rendered comparatively hydrophilic through the covalent bonding of e.g., the hydrophilic polymer polyethylene glycol to the polystyrene surface.

[0027] In a preferred embodiment, the method of the invention is practiced on a surface or other substrate that has not been pretreated. As used herein, the terms "pretreatment" and "pretreated" refer to the addition to a surface or other substrate of functional groups which are chemically involved in the covalent bonds subsequently formed upon activation of latent reactive groups. Substrates such as solid surfaces may be pre-washed, of course, to remove surface contamination and may be modified as desired to affect solvophilic characteristics without adding functional groups that are

involved in covalent bond formation with latent reactive groups. For example, polystyrene surfaces may be washed and then exposed to hydroxyl ions in known water vapor plasma contact procedures so as to add hydroxyl groups to the surface solely for the purpose of rendering the surface more readily wetted by aqueous solutions, the hydroxyl groups not being involved in subsequent covalent bond formation with the surface upon latent reactive group activation. Avoidance of pretreatment steps, as above defined, leads not only to important processing economies but also avoids technical problems associated with the attachment of bond-forming reactive groups to surfaces at uniform loading densities.

[0028] The invention may be more easily appreciated by reference to the following non-limiting examples, in which parts are expressed by weight unless otherwise indicated.

[0029] In the following Examples, the present invention is exemplified in Example VII. The remaining Examples I to VI, VIII and IX are provided by way of background information.

EXAMPLE I

Modification of the Surfaces of Contact Lenses and Introcular Lens Implants

[0030] The experiments described in this example involved preparations of hydrophilic polymers that are covalently bonded to contact lens surfaces through latent reactive groups carried by the polymers.

[0031] Preparation of Photolabeled Polyethylene Glycols. Polyethylene glycols of molecular weights 1000 (PEG-1000) and 4000 (PEG-4000) were labeled with fluoro-2-nitro-4-azidobenzene (FNAB) by modification of the phase transfer method of Kimura, and S. Regen. *Journal of Organic Chemistry* 48: 195 (1983). Briefly, the phase-transfer synthesis of 4-azido-2-nitrophenyl-polyethylene glycol (ANP-PEG) involved the mixture of 60% aqueous potassium hydroxide ("KOH")/toluene with FNAB and PEG, followed by extraction and thin-layer chromatographic (TLC) purification as described below.

[0032] ANP-PEG-1000. ANP-PEG-1000 was prepared by adding 0.3 mole PEG-1000 to 5 mls 60% KOH and 0.15 mmole FNAB to 10 ml toluene. This reaction mixture was rapidly stirred at room temperature for 16 hours. The product was isolated from the organic layer. TLC in 85/15/1/1 chloroform/methanol/H₂O/acetic acid or ammonium hydroxide separated mono- and di-substituted derivatives of ANP-PEG-1000 from unlabeled PEG. The band corresponding to ANP-PEG-1000 (lower R_f value) was extracted from silica gel with TLC solvent and azeotrophed to remove residual acid or base. The final product was soluble in water.

[0033] ANP-PEG-4000. ANP-PEG-4000 was prepared by the same procedure as that described above except that the reaction mixture was rapidly stirred at 50°C to ensure all reagents remained in solution during the course of the reaction.

[0034] Preparation of Photolabeled Jeffamines. Polyoxypropylenepolyamines and polyoxyethylenepolyamines (referred to as "Jeffamines", a trademark of Jefferson Chemical Co., Inc.) were photolabeled by coupling the N-oxysuccinimide ("NOS") esters of ANP-EACA (epsilon-aminocaproic acid), BBA (4-benzyl benzoic acid) and nBBA (4-(3nitrobenzyl)benzoic acid) to the polymers. These NOS-derivatives were added to a two molar excess of Jeffamine in very dry (high purity) solvents (ANP-EAC-NOS in dry tetrahydrofuran, BBA-NOS in dry dioxane or dimethylformamide and nitro BBA-NOS in dry dioxane or dimethylformamide). After 16 hours of reaction at room temperature in the dark, the products were isolated by TLC in 85/15/1/1/chloroform/methanol/H₂O/acetic acid. Monosubstituted Jeffamine derivatives were extracted with the TLC solvent and azeotroped with water to remove the residual acetic acid. The ANP-EAC-Jeffamine, BBA-Jeffamine, and nBBA-Jeffamine products were water soluble.

[0035] Preparation of ANP-Hyaluronic Acid. The terminal sugar of human placental hyaluronic acid (MW_{app} 100-130,000) was activated by the periodate procedure described in E. Junowicz and S.E. Charm, "The Derivatization of Oxidized Polysaccharides for Protein Immobilization and Affinity Chromatography," *Biochimica et Biophysica Acta*, Vol. 428: 157-165 (1976). This procedure entailed adding sodium or potassium periodate to a solution of hyaluronic acid thus activating the terminal sugar. The hyaluronic acid was added to a 10-fold excess of Jeffamine and allowed to react 4 hours at room temperature. The linkages were stabilized by reduction with sodium cyanoborohydride, followed by exhaustive dialysis to remove non-bound Jeffamine. A 10-fold molar excess of ANP-EAC-NOS in DMF was added to the Jeffamine-hyaluronate in 0.1 M carbonate, pH 9.0, by syringe drive. This addition required 16 hours and was conducted at room temperature in the dark. The excess ANP-EAC-NOS and ANP-EAC-Jeffamine was removed by gel filtration chromatography. The integrity of the azide group, which is required for photocoupling of the moiety to the contact lens polymer backbone, was analyzed by infrared spectroscopy to detect the azide function of the ANP group, a polyethylene glycol assay to detect the Jeffamine spacer, and a modified carbazole assay described in T. Bitter and H. Muir, *Analytical Biochemistry* Vol. 4: 330-334 (1962) to determine the uronic acid content of the derivative.

[0036] The polyethylene glycol assay was developed using the Dragendorff reagent (tetraiodobismuthic acid-barium chloride). A 5-ml portion of stock reagent (425-mg bismuth nitrate, 10-gm potassium iodide in acetic acid and water) was added to 10-ml 10% barium chloride in water and a background reading at 516/nm was noted. Then 0.1-ml of the sample was added and the contents mixed by inversion of the cuvette. A reading was taken at 516/nm after 1 minute of

incubation. The values were compared to a standard curve.

[0037] The carbazole assay was performed as follows. A 3.0 ml portion of sulfuric acid reagent (0.025M sodium tetraborate in sulfuric acid) was cooled to -70°C. A 0.5 ml portion of sample was layered onto the acid and the mixture was stirred (30 min.) until it reached room temperature. The tubes were heated at 100°C. (10 min.), a 0.1 ml aliquot of carbazole reagent (0.125% carbazole in absolute ethanol) was added, the tube contents were mixed (5 min.), heated at 100°C. (15 min.), then cooled to room temperature in an ice bath. The samples were analyzed spectrophotometrically at 530 nm against a sulfuric acid reagent blank. The results were compared to a standard curve constructed with 4-40 ug/ml glucuronolactone standards. The assay was sensitive to detecting 20 pmole of hyaluronic acid.

[0038] The fractions containing one ANP, one Jeffamine and one hyaluronate molecule were pooled and used.

[0039] Preparation of Photolabeled Hyaluronic Acid, Methyl Cellulose and Chondroitin Sulfate. Sodium hyaluronate was dissolved in deionized H₂O to give a 1% polysaccharide w/v solution. This 1% solution was then dialyzed against 0.05M MES (2-(N-Morpholino) ethanesulfonic acid), pH 4.0 to convert the sodium salt to the acid form of hyaluronic acid. A 0.135 mole portion of ANP-EAC-Jeffamine or BBA-EAC-Jeffamine solid was added to the 10-ml of 0.5% hyaluronic acid and a thorough mixture of the two reagents was made by rotary stirring for 30 minutes. To this mixture was added 190 mg of EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and 40 mg Sulfo-NHS (N-hydroxysulfosuccinimide) at thirty minute intervals for a total of four additions. Thirty minutes after the final addition, the volume was reduced by one-half by rotary evaporation and the reaction was left to continue an additional 12 hours at 4°C. After the 12 hours of incubation, the reaction mixture was transferred to a dialysis bag of 12,000 MW cut-off. The mixture was then dialyzed against four changes of deionized H₂O to remove non-covalently bound ANP-EAC-Jeffamine, followed by dialysis against saline containing 0.001% thimerosal preservative. This reaction product was then analyzed by the carbazole reaction to assess remaining uronic acid residues, and UV spectroscopy to quantitate the photogroup loading on the polysaccharide. A loading density of one out of every 20 carboxyl groups modified with photogroup was obtained by this process. Photolabeled carboxymethyl cellulose and chondroitin sulfate can be prepared by similar procedures.

[0040] Preparation of Photolabeled Collagen. Human placenta Type IV collagen (available from Sigma Pharmaceuticals) was dissolved at a 1 mg/ml concentration in 0.1M borate, pH 9.0. ANP-EAC-NOS in DMF, BBA-sulfo-NOS in dioxane or nitro BBA-NOS in dioxane was slowly added to the collagen solution in a 50x molar excess by syringe drive at 4°C in the dark over 16 hours. After the addition was complete, the mixture was stirred 4 hours in the cold. The collagen product was dialyzed against 4 changes of PBS then centrifuged to remove insoluble material. The supernatant was measured spectrophotometrically at 260 nm, 280 nm and 462 nm to assess the photogroup/protein ratio.

[0041] Preparation of Photolabeled Proteinases. ANP-EAC-NOS, BBA-NOS and nBBA-NOS photogroup dissolved in organic solvent at 25 mg/ml concentrations, were added in 50 molar excess to papain (papaya, MW 23,426) by syringe drive at 4°C in the dark over 16 hours. After addition of the photogroup was completed, the mixture was stirred an additional 4 hours, then dialyzed in PBS to remove uncoupled photogroups. After dialysis, the product was centrifuged to remove insoluble material. The supernatant was measured spectrophotometrically at 260 nm, 280 nm, and 462 nm to estimate the photogroup/protein ratio.

[0042] Photocoupling of Polymers to Lens Surfaces. The photolabeled polymer agents obtained above were added to the contact lens materials described in Table 4 at a concentration of 250-1000 pmole polymer/contact lens. The solution was allowed to adsorb onto the contact lenses at room temperature in the dark for 3 hours. The polymers were then covalently linked to the plastic by photolysis for 12 hours at the appropriate wavelength (450 nm for ANP and 320 nm for BBA and nBBA derivatives). After photolysis, the contact lenses were washed with 5x5 ml of normal saline (0.85% NaCl) to remove non-covalently linked groups.

[0043] Radiolabeled groups may be coupled to the lens materials, and the lens pieces treated with tetrahydrofuran followed by DMSO to release the radiolabel from the solid surface. Scintillation fluor is then added and the amount of polymer/cm² is determined by liquid scintillation spectroscopy. Representative results are shown in Table 1.

Table 1

Photolabeled Polymer	Contact Lens Material	ng/cm ²
ANP-PEG-1000	Polyvinyl chloride	19.96
	Sofspin (polymacon)	33.45
	Permaflex	33.97
	Vistamarc	34.26
	Lidofilcon	63.12
	Silicone	33.97
	**Polymacon Button	2408.60
ANP-PEG-4000	Sofspin (polymacon)	108.24
	Permaflex	171.44
	Silicone	682.40
	**Polymacon Buttons	6296.00
nitro BBA-PEG-2000	Polyvinyl Chloride	46.40
	Sofspin	26.28
	Permaflex	16.42
	Silicone	191.22
	**Polymacon Buttons	7476.00
BBA-PEG-2000	Silicone	226.40
	**Polymacon Buttons	8070.20
ANP-Hyaluronic acid	Silicone	25.00
	**Polymacon Buttons	130.95

Sofspin contacts are made of polymacon with about 38.6% water and are a trademarked product of Bausch & Lomb, Inc.

Permaflex contacts are made of poly(hydroxyethylmethacrylate) with about 74% water and are a trademarked product of Coopervision, Inc.

Vistamarc contacts are made of poly(hydroxyethylmethacrylate) with about 58% water and are a trademarked product of Johnson & Johnson.

Lidofilcon contacts are made of poly(hydroxyethylmethacrylate) with about 70% water and are a product of Bausch & Lomb, Inc.

* Values were averaged from replicates of 10

** Polymacon loads are based on total volume, cm³, rather than surface area.

[0044] The values in Table 1 are expressed as ng of polymer per square centimeter of surface. The ANP derivatives coupled at higher load densities than the nBBA-Jeff on the hydrogel contact lens materials. These results were reversed for the silicone compound.

[0045] In Vitro Protein Adsorption Studies. Artificial human tears were prepared according to the formula found in B.P. Gloor, "The Lacrimal Apparatus" in *Adler's Physiology of the Eye: Clinical Applications* (R.A. Moses, ed.), C.V. Mosby Co., St. Louis, MO (1981). As indicated in that reference the major proteins present in human tears are serum albumin (HSA), gamma-globulin (HGG), and lysozyme (LYZ). The major sterols present in human tears are cholesterol and cholesterol esters.

[0046] ³H Proteins. The protein components were tritiated by reductive methylation with formaldehyde and tritiated sodium borohydride as described in N. Jentoft and D. C. Dearborn, *Journal of Biochemistry*, Vol. 254: 4359-4365 (1979). Briefly, the proteins in 1mg/ml concentration in 0.1M HEPES, pH 7.4 were methylated by reacting with formaldehyde and tritiated sodium borohydride, rocking at 22°C for about 2 hours. The product was dialyzed against PBS in 0.01M phosphate, 0.15M sodium chloride, pH 7.4, and affinity purified on gelatin sepharose. Bound agent was eluted

with 1M sodium bromide 0.02M sodium acetate, pH 5.0, then dialyzed against PBS, pH 7.4.

[0047] Preparation of Artificial Tears. The radiolabeled proteins described above were used in preparation of artificial tears. One of the radiolabeled proteins or the tritiated cholesterol was included in each tear mixture. The other components were not radiolabeled. The contact lens materials were incubated in the artificial tear solution for one week at 37°C with gentle agitation. At the end of this time the lens materials were washed with 5 x 10ml of 0.85% NaCl. The amount of protein adsorbed to the lens materials was then determined by liquid scintillation counting.

[0048] Reduction in total protein deposition reached 85% in ANP-PEG-1000 modified Sofspin lenses. The overall protein amounts were reduced for all lens materials except ANP-1000-OH coated Polymacon buttons. ANP-4000-OH coated polymacon buttons and ANP-hyaluronate coated polymacon buttons. These poor results were all obtained with virgin polymacon materials which appears to react differently than polymacon contact lenses, such as Sofspin lenses. Overall, these *in vitro* protein deposition studies demonstrated significant to dramatic decreases in protein deposition from artificial tears on various contact lens materials during a one week period.

[0049] Amino Acid Analysis. Control and surface modified lenses were incubated in the artificial tear solution for one week at 37°C with gentle agitation. The lenses were washed with 5 10ml washes of 0.85% NaCl, then hydrolyzed with 6N HCl and the hydrolysates subjected to standard amino acid analyses on an amino acid analyzer. Total amino acid content of control and surface modified lenses were compared to each other. Reduction in total amino acid content indicated a reduction in protein absorption.

[0050] The total amino acid analyses of the acid hydrolyzed contact lenses are given in Table 2. These results are expressed as total amino acids in nmole/ml. These results again indicated that the ANP-PEG-1000, ANP-PEG-4000 and nBBA-Jeff modifications of Sofspin polymacon lenses reduced the deposition of proteins on the lenses after 7 days of incubation in artificial human tears.

Table 2

Total Amino Acid Analyses from Artificial Tear Deposits on Contact Lenses			
Contact Material	Biocompatible Agent	Total Amino Acids nmol/lens	% Reduction
Sofspin	ANP-PEG-1000	62.8	59.7
	ANP-PEG-4000	136	12.4
	nBBA-Jeff	105	32.3
	Control	156	----
Permalens	ANP-PEG-1000	169	32.5
	ANP-PEG-4000	210	15.9
	nBBA-Jeff	181	27.5
	Control	250	----

EXAMPLE II

Preparation of Monofunctional (Photoreactive) Polyethylene Glycol

[0051] Dihydroxy PEG 1450, 0.125 moles, was dissolved in 800 ml of toluene, followed by removal by distillation of 200 ml of solvent to provide azeotropic removal of water. Triethylamine, 0.143 moles, was then added, followed by the addition of 0.0625 moles of the acid chloride of 4-benzoylbenzoic acid (Aldrich B1,240-7). The mixture was stirred for 2 hours at room temperature. The crude reaction mixture was filtered through a Celite pad to remove the hydrochloride salt of triethylamine and the toluene was removed under reduced pressure.

[0052] The crude product was then dissolved in water (2 ml water/ 1 g product) and solid sodium chloride was added (50 mg NaCl/ 1 ml water). After adjusting the pH to 7 using solid sodium bicarbonate, toluene (1 ml/ 1 g product) and isopropyl alcohol (1 ml/ 5 g product) were added and the layers were thoroughly mixed. This extraction was repeated until no more disubstituted PEG appeared in the organic phase by TLC as outlined in Example I. The aqueous layer was then briefly evaporated under reduced pressure (5% volume reduction) to remove organic solvents. The aqueous solution was then saturated with sodium chloride and extracted with toluene (1 ml/ 1 g solution) until all monosubstituted PEG was removed. Typically seven to eight extractions are required for complete removal of the monosubstituted PEG. The combined organic extracts were washed with saturated sodium chloride to remove any residual

dihydroxy PEG and then were dried over anhydrous sodium sulfate.

[0053] The solvent was removed under reduced pressure and the product was redissolved in a small volume of toluene (1 ml toluene/ 5 g product). The product was precipitated by the addition of diethyl ether (5 times volume of toluene) and cooling on an ice bath. Final filtration and drying of sample gave a 48% yield of a white solid.

EXAMPLE III

Polyethylene Glycol-Coated Ocular Prostheses.

[0054] Ocular prostheses (artificial eyes) are generally made from poly methylmethacrylate. Ocular prostheses were inspected, cleaned and polished by an ocularist, and were then sprayed with a methanol solution containing 0.3% of the BBA-PEG-1450 derivative as described in Example II. The spray volume was just sufficient to completely wet the surface of the prostheses.

[0055] The BBA latent reactive groups were activated by UV radiation to form an excited state which abstracts a hydrogen from the acrylic substrate to covalently couple the PEG to the acrylic surface. This UV activation was carried out under an Electrolite Model ELC 4000 which illuminates the prostheses at 1.5 microwatts/square centimeter for a period of three minutes. The prostheses were cleaned to remove excess reagent and then returned to the patient.

[0056] When the prostheses were placed in the eyes of patients, the patients reported the initial sensation was "different", "more comfortable", "cool", and "feels like [their] natural eye". Patients have been able to distinguish the effect of the coating after three months of continuous wear.

EXAMPLE IV

Preparation of Succinimidyl 6-(4-benzoylbenzamido) Hexanoate.

[0057] The acid chloride of 4-benzoylbenzoic acid, 0.041 moles, was dissolved in 150 ml of toluene and to that mixture was added a solution of 0.041 moles of 6-aminocaproic acid in 125 ml of 1 N sodium hydroxide. The mixture was stirred vigorously for 45 minutes at room temperature during which time a precipitate formed. The product was then acidified with 125 ml of 1 N HCL and extracted with 3 X 150 ml of ethyl acetate. The combined organic extracts were dried over anhydrous magnesium sulfate. After removal of solvent, the final product was recrystallized from toluene - ethyl acetate to give an 84% yield of a white solid, melting range 106-109° C. A portion of the above amide product, 0.0147 moles; was dissolved in 100 ml of dry dioxane, followed by the addition of 0.0270 moles of N-hydroxysuccinimide. The reaction mixture was protected from moisture by a drying tube and was cooled to 0° C. A solution of 0.0270 moles of dicyclohexylcarbodiimide in 3 ml of dry dioxane was added over a 5 minute period. The mixture was then stirred overnight with slow warming to room temperature.

[0058] The dicyclohexylurea byproduct was removed by filtration and the dioxane was removed under reduced pressure. The product was diluted with 50 ml of ethanol and was evaporated again to dryness under reduced pressure to aid in the removal of the dioxane. The white solid was then recrystallized twice from ethanol to give a nearly quantitative yield of the activated ester, melting range 120-122° C.

EXAMPLE V

[0059] The compound of Example IV is reacted with tritiated Human Type IV collagen by the following method: Tritiated Type IV collagen (previously dialyzed against borate-buffered saline at a concentration of 4 milligrams per milliliter) is mixed 10/1 by volume with 1 Molar sodium carbonate (pH 9). The compound of Example IV is dissolved in dry dimethyl formamide at 6 milligrams per milliliter and 8.0 mls of the resulting solution is added (over three hours) via syringe drive to 28 mls of a gently stirred (4°C) solution of the Type IV collagen described above. The mixture is allowed to continue stirring for an additional hour. All material, including the precipitate that forms is dialyzed against several changes of phosphate buffered saline. The resultant reagent (which is insoluble) is suspended in deionized water and the pH is adjusted to 4 with dilute hydrochloric acid at which point the material goes into solution. The resultant reagent is applied to polystyrene tissue culture surfaces at a concentration of 13 ug of collagen/square cm and photoactivated in the same manner as the prosthesis in Example II. As a control, identical polystyrene tissue culture surfaces are treated with the same concentration of tritiated Human Type IV collagen without the photo groups attached. Both sets of surfaces are identically washed for 0.5 hour with five changes of a 0.1% solution of Tween 20 to remove unbound collagen.

[0060] The resultant washed surfaces are counted to show a several fold increase in retention by the photoderivatized collagen as compared to the control underivatized collagen.

[0061] Polystyrene tissue culture surfaces similarly prepared but using non-tritiated collagen are evaluated in actual

tissue culture using fetal bovine corneal endothelial cells. The tissue outgrowth is several fold greater than the control, indicating creation of a more tissue compatible surface.

EXAMPLE VI

[0062] The ability to repel proteins by surfaces is a desired goal for many blood compatibility applications (eg. in vivo blood sensors), and immuno gamma globulin (IgG) is a common blood protein which binds very tenaciously to plastics, especially polystyrene.

[0063] The BBA-PEG-1450 described in Example II, in a methanol solution, is sprayed into 96 well polystyrene microtiter plates at a concentration of 3000 ng of reagent per well. The methanol is allowed to evaporate off before the plates are photoactivated as described in Example II.

[0064] The wells are evaluated by absorbing Human IgG and evaluating the levels of absorption by standard ELISA methodology using an alkaline phosphatase enzyme coupled to anti-IgG. The PEG coated wells absorb only 20% of the IgG level of that absorbed onto untreated wells, indicating the ability to repel a very tenaciously absorbable blood protein.

EXAMPLE VII

[0065] 4-Benzoylbenzoyl-lysyl-acrylate (BBA-LYS-AC) is prepared by reacting N-epsilon-t-BOC-L-lysine with 4-benzoylbenzoyl chloride, then deblocking the epsilon-amino group with 3N HCl in ethyl acetate for one hour at room temperature followed by reaction with acryloyl chloride at pH 9.0. This reagent is coated onto silicone contact lenses by incubating the reagent solution in 50% ethanol in 0.1 M bicarbonate buffer at pH 9.5 for two hours at room temperature, then exposing to high intensity light at 305 nm. The acrylate is the polymerization site. A solution of 5% hydroxyethyl-methacrylate in 0.1M bicarbonate buffer at pH 9.0 is then polymerized on the lenses using 2,2 -azobisisobutyronitrile as catalyst. The polymerization reaction is allowed to proceed for one hour at room temperature, after which the uncoupled polymer is removed by washing the plates. This procedure improves wettability of the lenses.

EXAMPLE VIII

[0066] Hyaluronic acid was derivatized with the ANP-EAC-PEG-amine of Example I to result in a substitution level of one photo-group per 5 carboxyl groups on the polysaccharide. A 0.5% solution of this HA derivative was used to prepare a film of this material on glass or silicone materials. After application and drying, the film was cross-linked by exposure to high intensity light at 320 nm for three minutes. After photolysis, the film was lifted from the form by hydration with deionized water. The film maintained its integrity in water, was translucent, and retained mechanical stability allowing it to be picked up with a forceps and handled. The photochemical cross-linking of this material resulted in the construction of a film with greatly reduced rate of dissolution in aqueous systems.

[0067] This film has utility in wound healing and drug delivery in ophthalmic applications.

EXAMPLE IX

[0068] It is known that small polypeptide subunits of cell attachment factors such as those used in Example V are actually responsible for the cell response by activating specific cellular receptor sites. Immobilization of these subunits onto a surface should elicit responses similar to the actual cell attachment factors if they are so immobilized without significantly altering their chemical stereochemistry so that they retain the properties recognized by the cells.

[0069] The polystyrene tissue culture "slips" as used in example 5 were soaked for two hours in a solution containing 1.5 micrograms of the compound of Example 4 dissolved into 133 microliters of methanol. The slips are removed and the methanol solvent is allowed to evaporate in air. A solution of 200 micrograms of fibronectin peptide (FP) subunits per milliliter of 0.1 M bicarbonate (pH 9.0) in water was prepared. To one side of the polystyrene slip (0.5X1.0 cm) was added enough of the fibronectin peptide solution (75 microliters) to completely cover the entire surface. The slip maintained for 4 hours at 4°C. Without removing the solution the slip is photoactivated as in Example III for three minutes. The solution was next removed from the slip. The slip was then placed into 1.5 ml of 1.0% Tween 20 in phosphate buffered saline and allowed to rotate on an orbital shaker. The wash solution was replaced every thirty minutes for 1.5 hours, and then allowed to continue washing for approximately 16 hours before they were exhaustively rinsed with PBS. The slips were evaluated using corneal endothelial cell tissue culture as in Example V, against control slips having adsorbed FP and untreated control slips. The adsorbed FP and control slips showed minimal cell outgrowth and the immobilized FP exhibited 28 times the outgrowth of the control slips.

Claims

1. A method of providing a substrate having a solid surface with a polymeric coating, the method comprising the steps of:-

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(a) providing a monomer, oligomer or other reactive chemical unit, each having covalently bonded to it at least one latent reactive group capable of active specie generation in response to ultraviolet, visible or infrared electromagnetic radiation;

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(b) contacting the substrate surface with a composition comprising a plurality of molecules of the monomer, oligomer or other reactive chemical unit under conditions which enable the monomer, oligomer or other reactive chemical unit molecules to orient spatially so as to enable the latent reactive groups to come into covalent bonding proximity with the substrate surface;

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(c) thereafter activating the latent reactive groups by application of ultraviolet, visible or infrared electromagnetic radiation to covalently bond the monomer, oligomer or other reactive chemical unit molecules to the substrate surface; and

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(d) covalently bonding by polymerization further monomers or oligomers to the monomer, oligomer or other reactive chemical unit molecules covalently bound to the substrate surface.

2. A method according to claim 1 wherein the latent reactive group is selected from the group consisting of azides and photoactivatable ketones.

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3. A method according to claim 2 wherein the latent reactive group is an photoactivatable ketone.

4. A method according to claim 3 wherein the latent reactive ketone is derived from 4-benzoyl benzoic acid.

Patentansprüche

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1. Verfahren zum Bereitstellen eines Substrats, welches eine feste Oberfläche mit einem Polymerüberzug aufweist, wobei das Verfahren die Schritte umfaßt:

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(a) das Bereitstellen eines Monomers, Oligomers oder einer anderen reaktiven chemischen Einheit, wobei jede mindestens eine an sie kovalent gebundene latent reaktive Gruppe aufweist, welche befähigt ist, als Reaktion auf eine Bestrahlung mit ultravioletter, sichtbarer oder infraroter elektromagnetischer Strahlung, eine aktive Spezies zu generieren,

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(b) das Kontaktieren der Substratoberfläche mit einer Zusammensetzung, umfassend eine Vielzahl von Molekülen des Monomers, Oligomers oder der anderen reaktiven chemischen Einheit, unter Bedingungen, welche es dem Monomer, Oligomer oder Molekülen der anderen reaktiven chemischen Einheit ermöglichen, sich räumlich derart auszurichten, daß es den latent reaktiven Gruppen ermöglicht wird, mit der Substratoberfläche in die Nähe einer kovalenten Bindung zu gelangen,

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(c) danach das Aktivieren der latent reaktiven Gruppen durch Einsatz ultravioletter, sichtbarer oder infraroter elektromagnetischer Strahlung, um das Monomer, Oligomer oder Moleküle der anderen reaktiven chemischen Einheit kovalent an die Substratoberfläche zu binden, und

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(d) das kovalente Binden durch Polymerisieren weiterer Monomere oder Oligomere an das Monomer, Oligomer oder Moleküle der anderen reaktiven chemischen Einheit, welche kovalent an die Substratoberfläche gebunden sind.

2. Verfahren nach Anspruch 1, wobei die latent reaktive Gruppe aus der Gruppe, bestehend aus Aziden und photoaktivierbaren Ketonen, ausgewählt ist.

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3. Verfahren nach Anspruch 2, wobei die latent reaktive Gruppe ein photoaktivierbares Keton ist.

4. Verfahren nach Anspruch 3, wobei das latent reaktive Keton von 4-Benzoylbenzoesäure abgeleitet ist.

Revendications

1. Procédé pour obtenir un substrat ayant une surface solide munie d'un revêtement polymère, le procédé comprenant les étapes consistant à:

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(a) se procurer un monomère, un oligomère ou un autre motif chimique réactif, chacun étant lié par covalence à au moins un groupe réactif latent capable de produire une espèce active en réponse à un rayonnement électromagnétique ultraviolet, visible ou infrarouge;

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(b) mettre en contact la surface du substrat avec une composition comprenant une pluralité de molécules du monomère, de l'oligomère ou de l'autre motif chimique réactif, dans des conditions permettant aux molécules de monomère, d'oligomère ou d'autre motif chimique réactif de s'orienter dans l'espace de manière à permettre aux groupes réactifs latents de venir à proximité, pour permettre une liaison covalente, avec la surface du substrat;

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(c) activer ensuite les groupes réactifs latents en appliquant un rayonnement électromagnétique ultraviolet, visible ou infrarouge pour lier par covalence les molécules de monomère, d'oligomère ou d'autre motif chimique réactif à la surface du substrat; et

(d) lier par covalence, par polymérisation, d'autres monomères ou oligomères aux molécules de monomère, d'oligomère ou d'autre motif chimique réactif liées par covalence à la surface du substrat.

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2. Procédé selon la revendication 1, dans lequel le groupe réactif latent est choisi dans le groupe constitué par les azides et les cétones photoactivables.

3. Procédé selon la revendication 2, dans lequel le groupe réactif latent est une cétone photoactivable.

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4. Procédé selon la revendication 3, dans lequel la cétone réactive latente est dérivée de l'acide 4-benzoylbenzoïque.

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